Studies in the conjugation of Spirogyra ternata

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(with three text figures)

The discovery of but four chromosomes in *Spirogyra ternata* led to the collection of material in fruiting condition during the months of October and November, 1915 and 1916. The alga was growing under more or less unnatural conditions in a basin where the water was drawn off during the winter months. Its continued propagation seems uncertain as no trace of it was found in the fall of 1917 or 1918.

The large size of the nuclei, the low number of chromosomes, the few chromatophores, and the general transparency of the cells, combined with marked sexual differentiation of the filaments in conjugating periods, are features that make this species especially favorable material for the investigation of such debated questions as the potential bisexuality of vegetative cells, chromosome reduction, and the history of the secondary nuclei in the sporophyte generation. While material was at first studied with the hope of elucidation of these problems, continued study of the filaments brought to light interesting changes in the cells as they pass from the vegetative condition to a state of conjugation.

The diagnostic features of *S. ternata* Ripart (1876), as described by Collins (**2**, p. 114), are as follows: filaments 50–65 μ in diameter, with cells 1½–2 diameters long, somewhat swollen at the middle; zygospores ovoid, 45–66 μ in diameter, 1–1½ diameters long.

The form collected in 1915 varied from this in having its vegetative cells 74–80 μ in width. The zygospores in this 1915 material measured 64 μ in width and 120 μ in length. In the 1916 material there was much greater variability and frequent hybridization with a larger species resembling *S. jugalis* but differing in the shape of the fertile cell. The comparison of *S. ternata* with another species, *S. maxima*, growing under the same conditions and fruiting at the same season, revealed such great

differences in the form of the conjugating tubes and cell contents as to suggest the problems investigated in this paper.

A sinuosity is to be noted in the filaments in both vegetative and fruiting states, associated with a certain degree of motility. It is questionable which is the greater contributory factor to these movements, inequalities of growth or inequalities in the turgor of the cells, increased possibly by the mucilaginous layer on the cell wall. When in a state of conjugation the short portions of threads of *S. maxima* which are contained in the field of view under the low power of the compound microscope can be seen to be straight and to consist of large cells joined by small and narrow tubes. The fertile cells of this species are always unswollen. In *S. ternata* straight threads are rarely to be seen and then only in the earliest stages of conjugation, while a marked feature of the species is found in the swollen ♀ cells. The swelling often affects the cells throughout their entire length and sometimes increases their volume twofold, if the extensions of the conjugating tubes are taken into account. The filaments of *S. ternata* not only show a notable sinuosity, but in the curvatures of the filaments the two sexes always have a definite relation to each other, in spite of the fact that there are certain irregularities in the number of the cells. A comparison of numerous threads, whether lying in water or preserving fluids, showed that the filaments always assumed curves which varied from those slightly bent to those forming semicircles. Further examination disclosed the fact that the ♀ threads were invariably lying on the outer side of the curve, unless displaced or twisted in mounting. Often, in the water, the conjugating threads would naturally twist, but in so doing the ♀ thread, after a turn of a few cells, would again be found lying outside the ♂ filament.

S. ternata is an example of typical scalariform conjugation, for in the hundreds of filaments studied the movement in the conjugating cells was always found to be in one direction. It would be of interest to discover cases of cross conjugation, such as those reported by Cunningham (3) in a smaller species of *Spirogyra*. If forms of cross conjugation should be found, where regular alternating movements of contents take place, cells with straight outlines would seem probable, while if threads were found

containing several cells of one sex and then several cells of the opposite sex a zigzag position of the threads might be expected, instead of the symmetrical curves found in such a typically dioecious form as *S. ternata*.

It would seem that a continuous movement of contents of the ♂ cells in one direction, thereby increasing the contents of the ♀ thread as the zygospores form and subjecting it to a greater geotropic stimulus, would cause a sagging of the thread, resulting in its taking an outer or lower position in reference to the ♂ filament. Yet, in the majority of cases where the curvature was extreme and where the ♀ thread uniformly kept its position, there had been no appreciable movement of the contents, even when a complete formation of the conjugating tubes had taken place. This was evidenced by the fact that the chromatophores were unrelaxed in position and also by the intact connecting walls of the conjugating tubes. Since a large amount of conjugating material was available it seemed desirable to scrutinize more closely this relation of filaments in conjugation. It seemed possible that the degree of curvature might be dependent upon the amount of tumidity of the cells and not upon the degree of advancement in the formation of the zygospores. It was hoped also to bring to light more information as to the number and nature of the sexually potent cells and the manner of formation of the conjugating tubes.

Camera drawings were made of many pairs of filaments, in some cases numbering over fifty cells. A magnification of 140 diameters was used in order to have as many cells as possible in the field of view and yet to see clearly the position of the nuclei and chromatophores. Measurements of the drawings for length of the cells and filaments were obtained by two methods. In the first method a piece of thread held without undue tension, following the curving outer walls of the cells, was measured with the metric ruler. Slight discrepancies in this method would be due to the varying tension of the measuring thread. The other method employed, when it was desired to compare cell with cell instead of filaments as a whole, was to measure the distance from one transverse wall to the next with the ruler, not allowing for the convexity of the lateral walls. Such distances added would bring

out changes in the length of the filament as a whole but would not take into account differences in the two sides of the cell and in the extent of the cell walls.

For the ascertaining of these relations the two sides of the cell in each sex were measured in some of the most curved examples. The distance between the ends of the transverse walls opposite to the conjugating tube was taken in each case for measurement, as the various degrees of bulging of the inner walls where the tube emerged made accuracy difficult. When accurate measurements were needed to determine the size and position of the various cell organs, such as the nuclei and pyrenoids, higher powers and the ocular micrometer were used. Measurements of the drawings by means of the thread following the curving lateral walls always gave figures in excess of the sum of the lengths of the cells measured individually. Both of these methods, when so many hundreds of cells were examined, involved far less labor than the use of the ocular micrometer and gave the same relative proportions in the lengths of the cells.

The various measurements obtained in each series of observations have been arranged in tables. If a longitudinal series of cells is considered an individual plant, only a portion of a plant was measured in each case. It would, in fact, be impossible to be sure in collecting material that an entire thread, resulting from the germination of a zygospore, had been obtained. In this work, however, the cells of the *Spirogyra* have all been held as potentially the same.

Series A (FIG. 1) represents a pair of threads chosen for study by reason of their great convexity and torsion. In this series none of the opposing cells had apparently mingled their contents, and the nuclei are to be seen in their central positions with the chromatophores unrelaxed and contiguous to the cell wall, as in ordinary vegetative cells. In mounting these threads in the liquid on the slide the curvatures between *A* and *B* and between *C* and *D* in the figure projected much above the surface. Though a cover-glass was carefully lowered upon them the torsion was such that the flattening of the surface caused a breaking of the ♂ thread of the pair at *D*, one of the most curved points. Camera drawings were made of the threads as they were thus mounted,

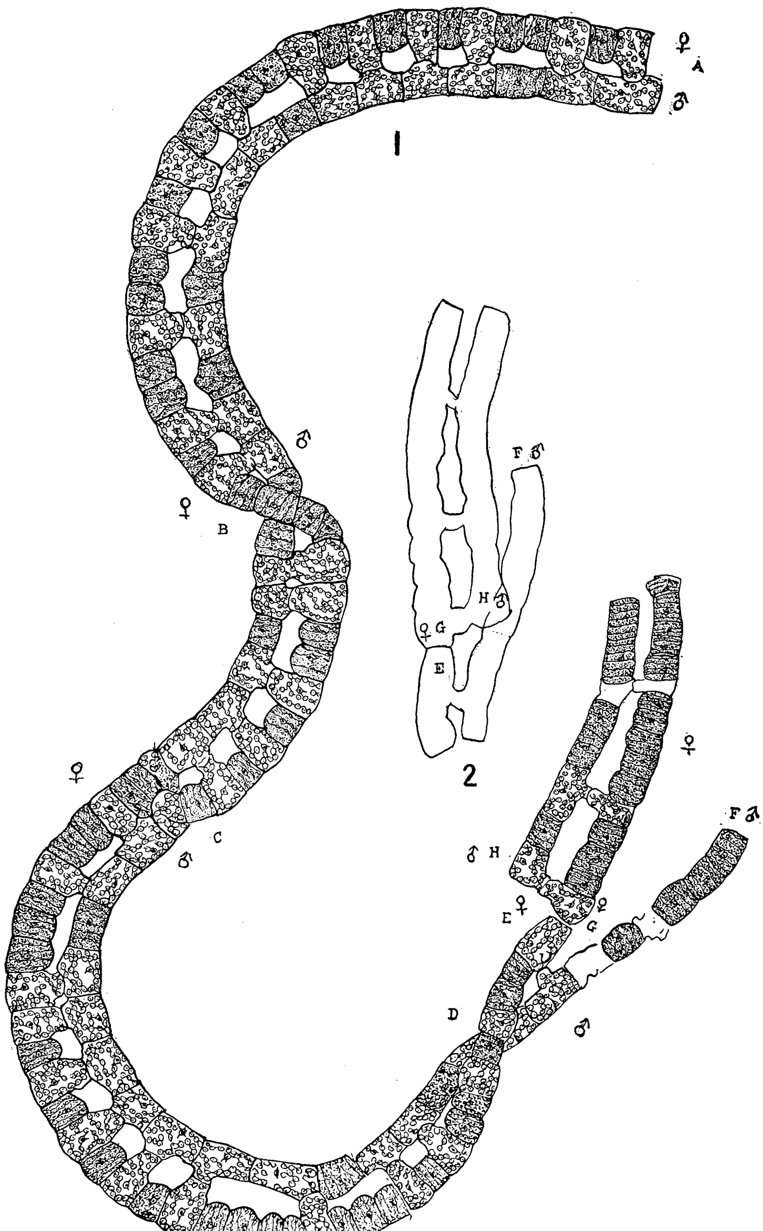

FIG. 1. A pair of conjugating filaments, showing torsion at points *B*, *C* and *D*; the ♂ thread is broken at *F*. × 140, reduced one half.

FIG. 2. A model of the broken filament replaced in its original position, showing relaxation in ♂ cells thatwere previously compressed.

the broken portion (upon lowering the coverglass) being reversed from its original position. FIG. 1 shows these threads as drawn originally, while FIG. 2, made by reversing a model of the broken threads, shows them as they were in their first position except for the break. The rupture of the thread reveals the previous tension of the cells. Upon severing the ♀ thread at *G* and then placing the terminal cell *H* of the ♂ thread next to the cell *F* from which it was broken, the ♀ thread, by reason of the relaxation of the ♂ cells in the breaking of the thread, lacks 45 mm. of meeting the cell *E* in the figure. When, on the contrary, the cell *G* is placed next to *E*, as in the model of the reversed cells, the thread shows a marked linear extension, indicated by the distance from *F* to *H*, the point to which *F* was previously joined. When this extension is measured by adding the distances between transverse

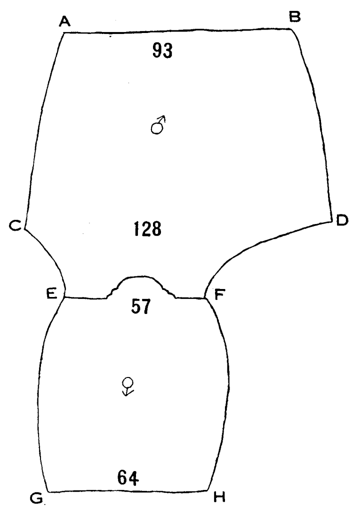

FIG. 3. Diagram of conjugating cells, illustrating distances between transverse walls as measured with the ocular micrometer.

walls it gives an excess of 43 mm.; when measured by a thread allowing for the curving walls, it gives an excess of 55 mm. These figures are an index of the amount of compression of the ♂ thread at this region of curvature.

The ♀ thread in this curved series, as shown in TABLE I, consists of ninety-five cells; thirty-six of these are conjugating

cells with their tubes formed, an equal number show by their tumidity an approach to the conjugating state, while only twenty-three show no signs of conjugation. Since there are only fifty-six cells all told in the ♂ thread it is obvious that many of the tumid ♀ cells would have proceeded to abortive conjugation. In the two portions of the filaments, *A–B* and *C–D*, where the curves are greatest and where in consequence the ♂ thread is under the greatest compression, it should be noted that the ♀ cells which show tumidity are opposite ♂ cells which have already conjugated. Seven conjugating cells in succession are included in the semicircular curve *C–D*, while in the curve *A–B* there is a succession of

TABLE I

SERIES A

Sex	Number of cells	Length of drawing of magnified filaments in mm. measured by thread	Sum of magnified lengths of cells, measured with ruler	Conjugating cells		Cells tumid, conjugating tubes not formed		Non-conjugating cells		Ratios	
				Number	Average length, microns	Number	Average length, microns	Number	Average length, microns	Potent cells (incl. tumid)	Non-potent
♀	95	779	701	36	63	36	48	23	42	.757	.242
♂	56	612	603	36	84	4	62	16	65	.714	.285
Excess.....	♀ 39	♀ 167	♀ 98		♂ 21	♀ 32	♂ 14	♀ 7	♂ 23	♀ .043	♂ .043

twelve conjugating or tumid cells with a single interruption. It is to be noted that the compression in the ♂ filament occurs before the rupture of the tubes and the mingling of the cell contents and hence before the relaxation of the chromatophores. In TABLE II the cells of Series A are grouped according to the length of the outer walls.

The next measurements taken were those of the distances between the outer and inner terminal points of the transverse

TABLE II

CELLS OF SERIES A ARRANGED ACCORDING TO LENGTH OF OUTER WALL

Length outer wall in microns	101–108	94–101	87–94	80–87	73–80	66–73	59–66	52–59	45–52	38–45	31–38	24–31	17–24
♀ conjugating..............			1		1	14	9	5	3	1	2		
♂ conjugating..............	2	6	4	5	14	2	2				1		
♀ tumid....................							2	10	10	7	6		1
♂ tumid....................					2	1				1			
♀ non-potent...............								3	7	7	3	2	1
♂ non-potent...............					4	3	3	3	2	1			

walls in each sexual cell. The significance of these measurements can be seen more clearly by taking as an illustration a single pair of cells, as in the accompanying FIG. 1. A ♂ cell, *ABCD*, has an outer wall, *AB*, measuring in a straight line 93 μ between the points *A* and *B*, this distance being less by 35 μ than a similar measurement in a straight line between the points *C* and *D* of the inner wall, from which the conjugating tube emerges. If the walls were originally an equal distance apart, as is the case in filaments where all the cells are non-conjugating and where external conditions are such as to cause no marked inequalities of growth in the two sides of the cells, *A* and *B* of the ♂ cell are brought nearer together, as conjugation proceeds, while *C* and *D* are spread farther apart. Internal pressure, the result of the metabolic activities of the protoplast, must cause this distension of *CD*. In the ♀ cell there is either no change whatever in the distance of *G* from *H* or only a slight increase. A study of the

TABLE III

CELLS A—B INCLUSIVE

LENGTHS GIVEN IN MICRONS

Number of ♀ cell	Length outer wall	Length inner wall	Outer wall increase	Conjugating with	Number of ♂ cell	Length outer wall	Length inner wall	Outer wall decrease
1	64	61	3	⟷	1	107	114	− 7
2	50	50	0					
3	71	71	0	⟷	2	100	96	+ 4
4	43	43	0		3	82	86	− 4
5	53	50	3					
6	64	64	0	⟷	4	79	89	−10
7	43	36	7					
8	57	50	7	⟷	5	79	79	0
9	61	57	4					
10	57	57	0	⟷	6	79	86	− 7
11	50	50	0					
12	71	64	7	⟷	7	71	86	−15
13	57	57	0		8	64	71	− 7
14	50	50	0					
15	64	64	0	⟷	9	71	86	−15
16	64	57	7					
17	79	57	22	⟷	10	107	129	−22
18	50	50	0					
19	79	71	8	⟷	11	100	107	− 7
20	64	64	0					
21	61	50	11		12	50	71	−21
22	79	71	8	⟷	13	86	100	−14
23	64	50	14		14	79	79	0
24	64	57	7		15	79	100	−21
25	71	71	0					
26	43	36	7					

membranes joining the completely formed conjugating tubes shows that the center of the tube pushes against the wall, forming a secondary smaller tube shown in the diagram. Probably it is the stretching of the wall as this tube presses against the wall of the cell that brings E and F nearer together.

Inspection of the TABLES III and IV, representing measurements of the cells where the curves in the filaments were greatest,

TABLE IV

CELLS C—D INCLUSIVE

LENGTHS GIVEN IN MICRONS

Number of ♀ cell	Length outer wall	Length inner wall	Outer wall increase	Conjugating with	Number of ♂ cell	Length outer wall	Length inner wall	Outer wall decrease
1	61	50	11	⟷	1	86	100	−14
2	43	36	7		2	79	100	−21
3	57	50	7					
4	64	50	14					
5	71	71	0	⟷	3	79	100	−21
6	57	57	0	⟷	4	79	86	− 7
7	43	43	0					
8	71	64	7	⟷	5	100	114	−14
9	50	50	0					
10	64	64	0	⟷	6	71	79	− 8
11	57	50	7					
12	64	57	7	⟷	7	93	107	−14
13	57	43	14					
14	71	64	7	⟷	8	100	107	− 7
15	50	43	7					
16	57	57	0					
17	42	42	0					
18	57	28	29					
19	57	50	7	⟷	9	111	128	−17
20	35	35	0		10	50	86	−36
21	50	50	0					

A—B and *C—D* inclusive, show the opposing numerical relations between the outer and inner walls of the two series.

From the comparison of the increases and decreases in the lengths of the walls in the two filaments a marked tendency is apparent toward lengthening the inner distance between the transverse walls of the ♂ cells, the corresponding distance between the transverse walls of the ♀ cells remaining the same or showing a slight decrease. The changes in length are brought about just before and during the process of conjugation. There is no reduction to be seen in any of the outer walls of the ♀ cells in these series, their excess lengths varying from zero up to 22 μ. In the case of the seventeenth cell, where there is the unusual in-

crease of 22 μ, the corresponding cell of the ♂ filament shows a reduction of 22 μ in the outer wall. This equality is presumably a coincidence, since the second ♂ cell in TABLE IV shows a similar reduction of 21 μ in the outer wall. This cell is not conjugating but is opposite tumid cells of the ♀ thread and is probably influenced by them.

In the ♂ filament there is a reduction in the outer wall in every cell but the second from the end *A*. In this cell it is to be seen that more of the wall than is usually the case takes part in the formation of the tube. All of this wall is elevated from the level of a straight line connecting the transverse walls. The bulging of this wall has probably counteracted the usual tendency of the inner parts of the transverse walls to spread apart.

According to these measurements, then, the compression in the filament, as revealed in the breaking and subsequent replacement of the broken parts, does not involve the filaments as a whole but is restricted in its extent to the parts of the cells which are farthest from the conjugating tube. Measurements also indicate that differences in tension in the ♀ cells are not so different from those occurring in vegetative cells. In this connection it is to be remembered that all the cells of a filament are in a state of slight compression, for when a filament is broken or an intervening cell dies the end cell extends slightly, rounding off and assuming the regular shape of an ordinary terminal cell. This does not explain the striking and opposing differences in tension found exclusively in cells in the conjugating state.

In another part of this investigation other changes in the conjugating cells will be considered, especially the formation of the tubes. There it will be noted that more of the inner wall of the ♀ cell takes part in the formation of the tube than is the case with the ♂ cell. Hence, as in FIG. 3, where *E* and *F* of the ♀ cell are always nearer relatively than *C* and *D* of the ♂ cell, the linear extension of the conjugating tube of the ♀ cell will be found to exceed that of the ♂ cell, if all of the linear extension pressing against the opposing wall is considered. However, it is the bringing together of the transverse walls and the consequent rectilinear contraction of one side of the filament at the beginning of conjugation that results in the constant relative positive of the

two sexes. This relative position was even maintained when *S. ternata* conjugated with the much larger species present. Whether a large ♀ cell of this species united with a small ♂ cell of *S. ternata* (the two differing by 32–50 μ in cell width), or whether a large ♂ cell conjugated with a smaller ♀ cell of *S. ternata*, this same relative position of sexes was maintained. The ♀ thread, whether larger or smaller, was always seen to occupy the outer position in the curve.

Studies will be presented also of the curvatures maintained in cases of triple and quadruple combinations of threads. Many examples were studied where one ♂ filament is conjugating with two ♀ filaments and, reciprocally, where one ♀ filament is conjugating with two ♂ filaments. Frequently a ♀ cell was found conjugating successfully with a ♂ cell on one side while the ♂ cell on the other side, though the conjugating tube had formed, showed only abortive conjugating. In the case of such an abortively conjugating cell it is important to note that the constant and marked inequalities in the distances of the transverse walls, seen in normally conjugating ♂ cells, were never exhibited. Except for its projecting tube the abortively conjugating ♂ cell, in the measurements between its transverse walls, accords more with those of the of the normally conjugating ♀ cell than with those of the normally conjugating ♂ cell. Abortive conjugation in *Spirogyra* has been previously reported by Bessey (1), Robertson (5) and, more recently, by Cunningham (3).

The work of Riddle (4) on the nature of sex in pigeons has a suggestive bearing upon these observations made on conjugating *Spirogyra*. He states, as a result of his extensive experiments, that sexual differentiation is to be interpreted as the expression of quantitative differences in the rate of protoplasmic activities, the more active metabolism resulting in males. With a low level of metabolism in the female is associated large size of yolk, low percentage of water in the yolk, high percentage of stored material, and a high total of stored energy. His review of the literature shows evidence of a relation between the rate of metabolism and sex in a great variety of animals, ranging from worms to man. Although his work and conclusions relate only to the animal kingdom he makes the conjecture that it would be of interest to

determine whether in dioecious plants there is a corresponding difference in the rate of metabolic exchange.

Since the ♂ gamete of *Spirogyra*, in contrast to the spermatozoön of animals, carries with it to the receiving gamete stored food as accretions about the pyrenoids, a high percentage of stored material does not occur in the ♀ cell until after conjugation is completed. The large proportion of swollen cells, both with and without conjugating tubes, and the fact that no visible movement of solid parts has as yet begun in the conjugating cells indicate that the different tensions shown by the measurements of the sex cells are to be causally connected with the differing amounts of tumidity and this in turn with different types of metabolism in the sex cells.

It would be premature to draw final conclusions from the intensive study of but one series. The comparison of Series A with other series observed, the tabulation of results and deductions as to potency of cells, formation of tubes and other related problems must be reserved for another paper.

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